Enhanced and multiplexed detection via a novel RCA-aptamer based sensing system*

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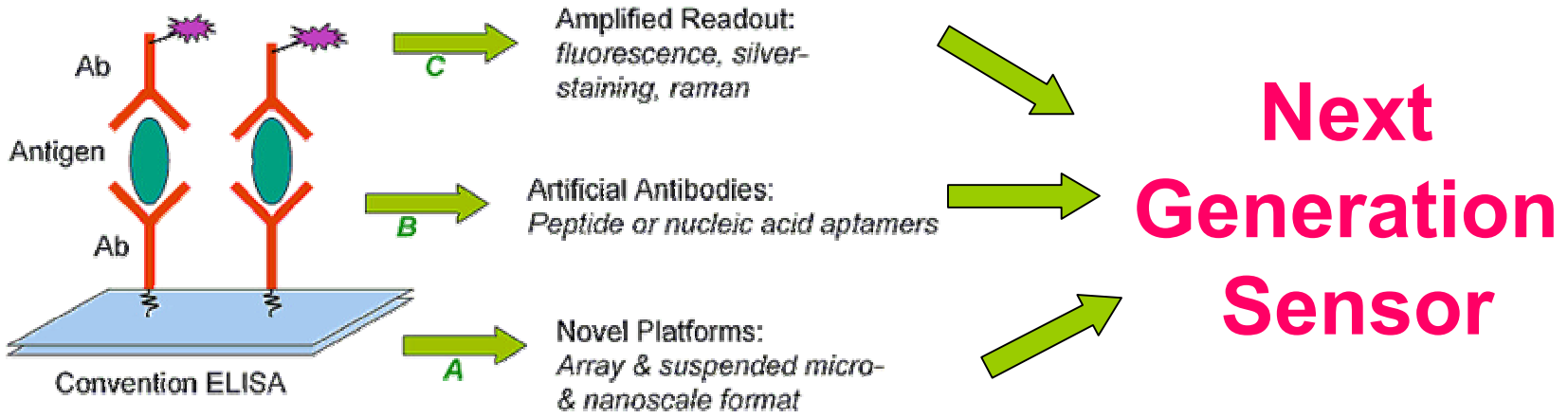
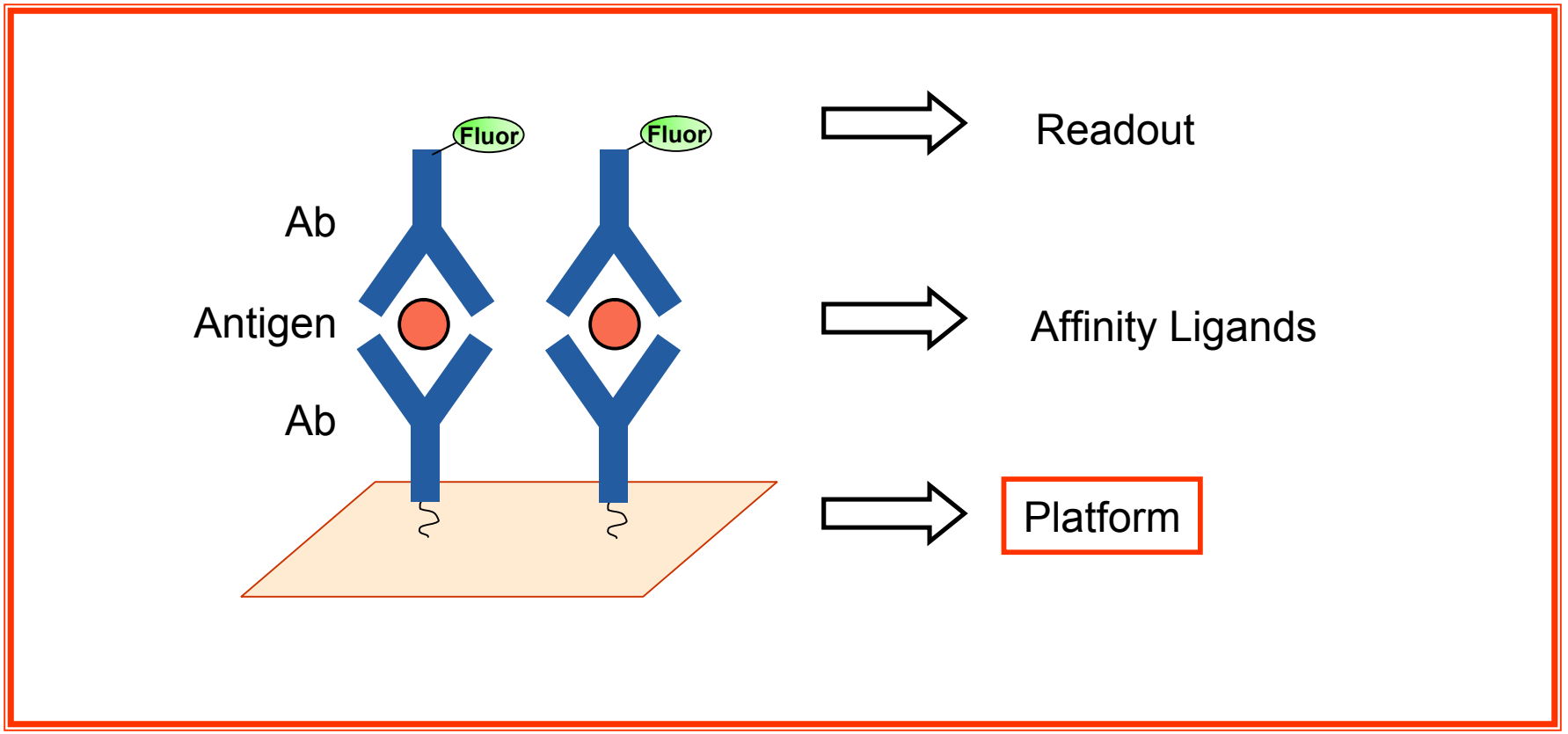
Research activities in our lab aim to enhance the following three major component essential for both *bio*- and *chem-detection*.



We have developed multi-striped metallic nanowires as platform for multiplexed sensing



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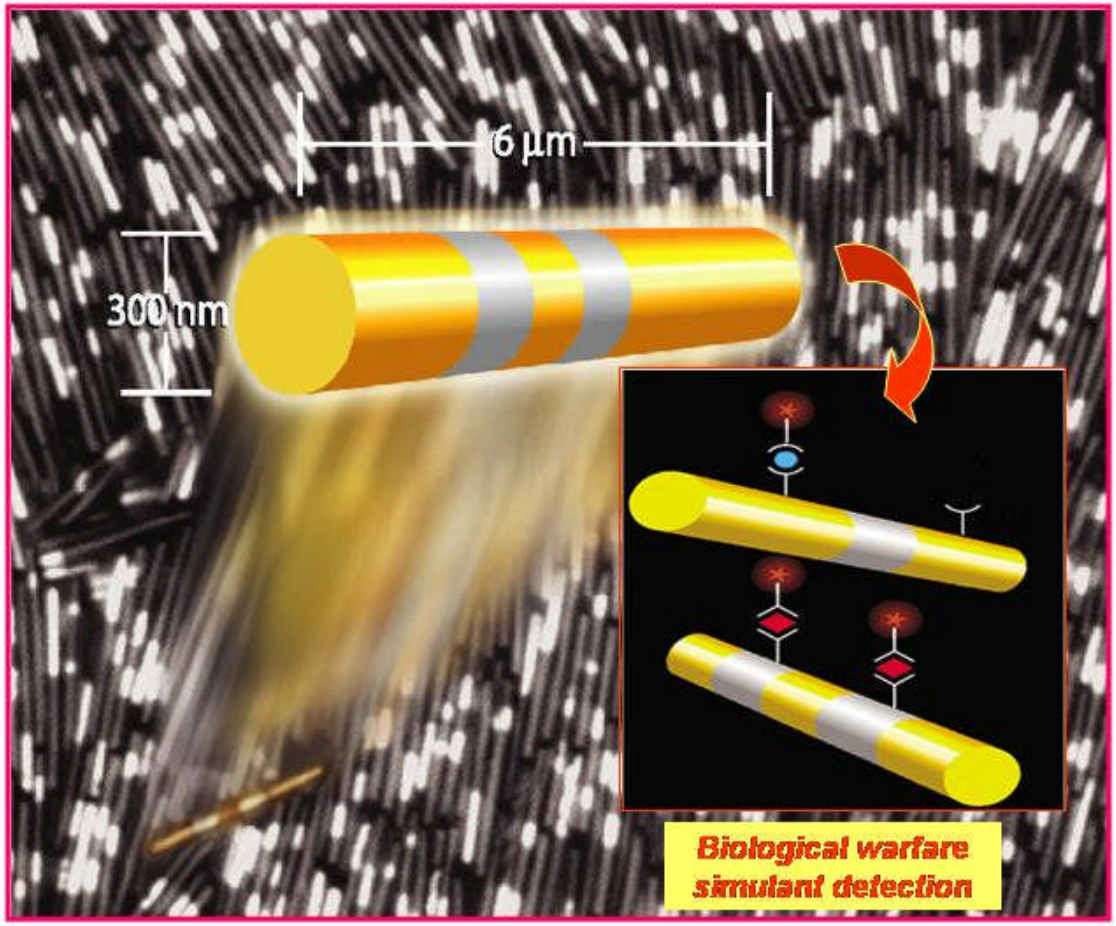
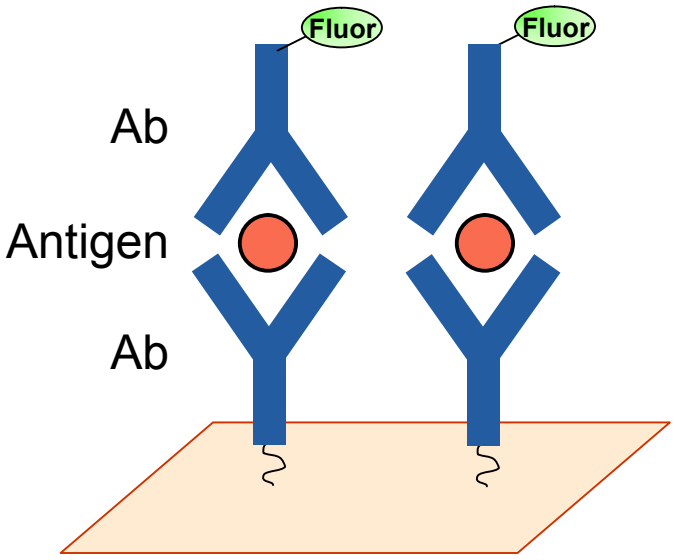


J. Tok et al., *Angew Chemie Int Ed*, **2006**, In Press

Our goal is to adapt new technologies by integrating recognition and detection of biological and chemical targets

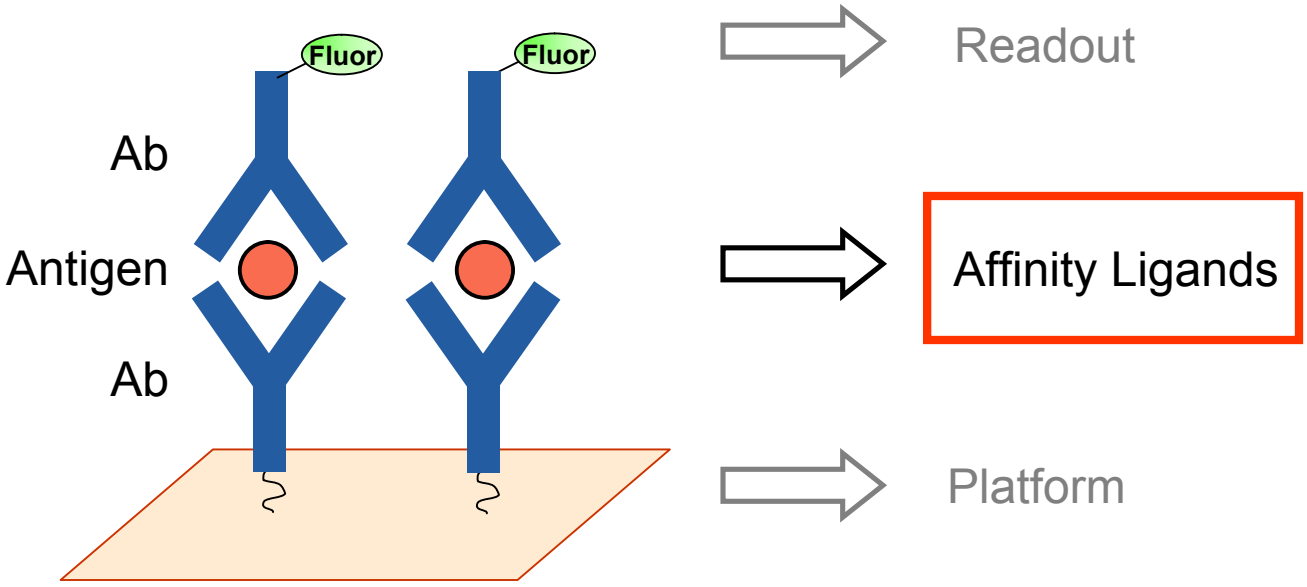


Readout

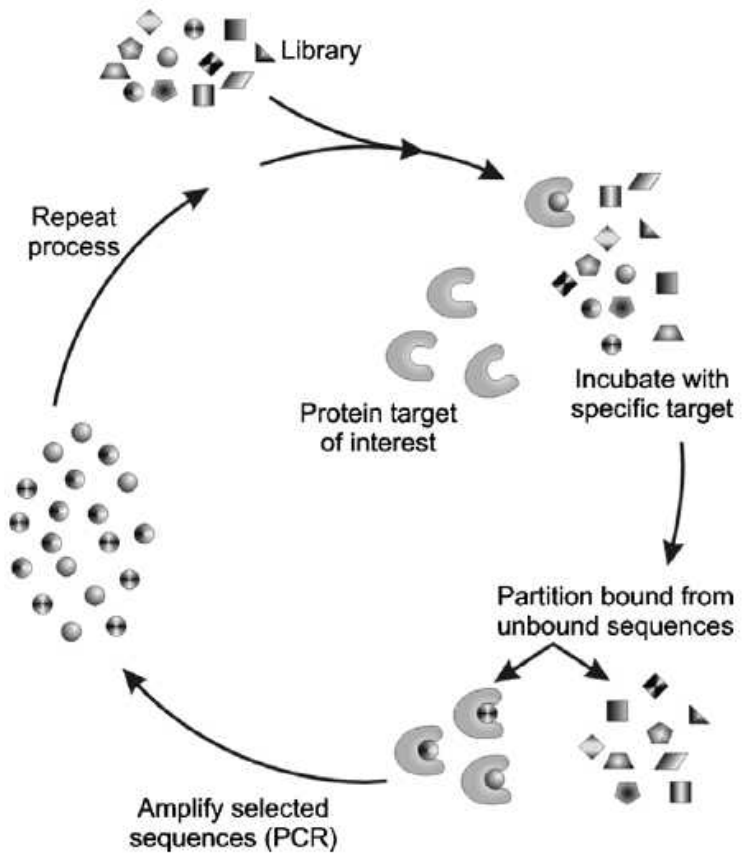
Affinity Ligands

Platform

One of our lab's major focus is to use DNA aptamers as affinity ligands



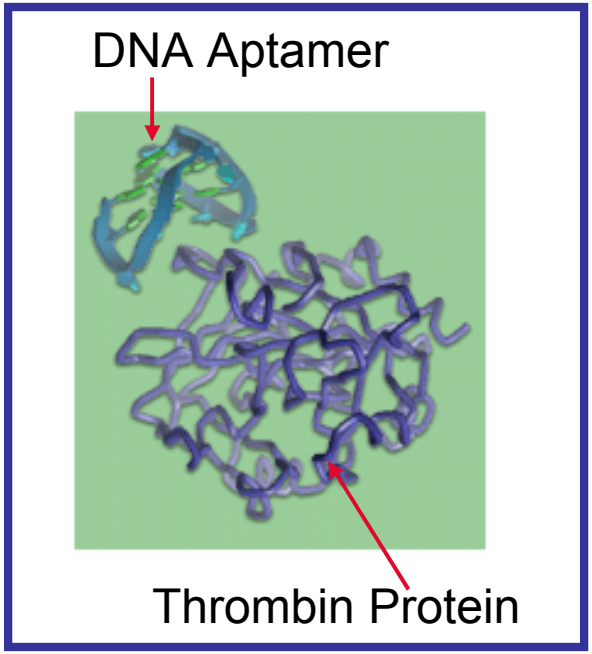
Systematic Evolution of Ligands by Exponential Enrichment (SELEX) is used to generate target binding ssDNA aptamers



Guthrie et al, 2005

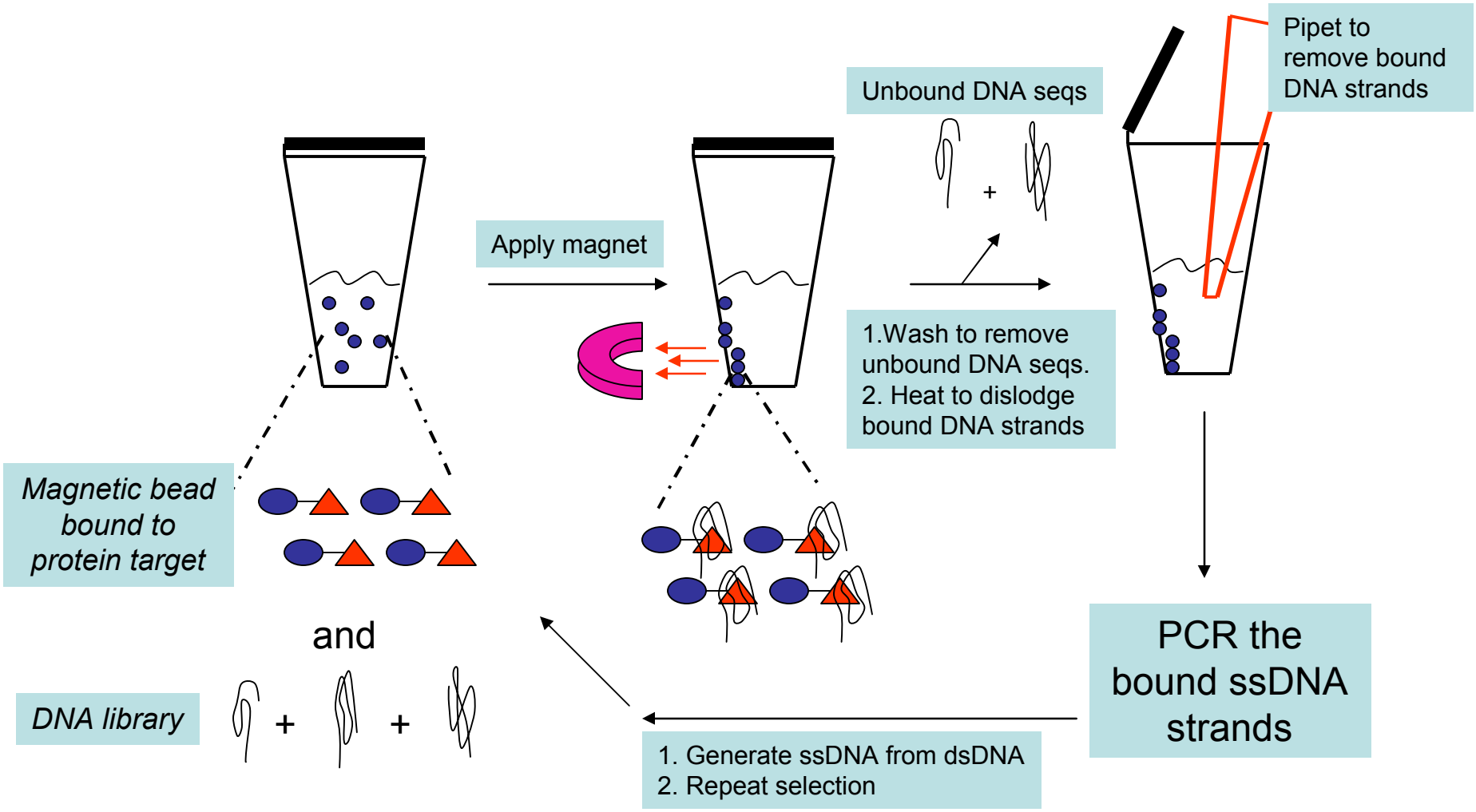
Unique characteristics

- **Selected against difficult targets**
- **Easily functionalized/tailored**
- **Easily fabricated**
- **Avoids batch variability found with polyclonal antibodies**



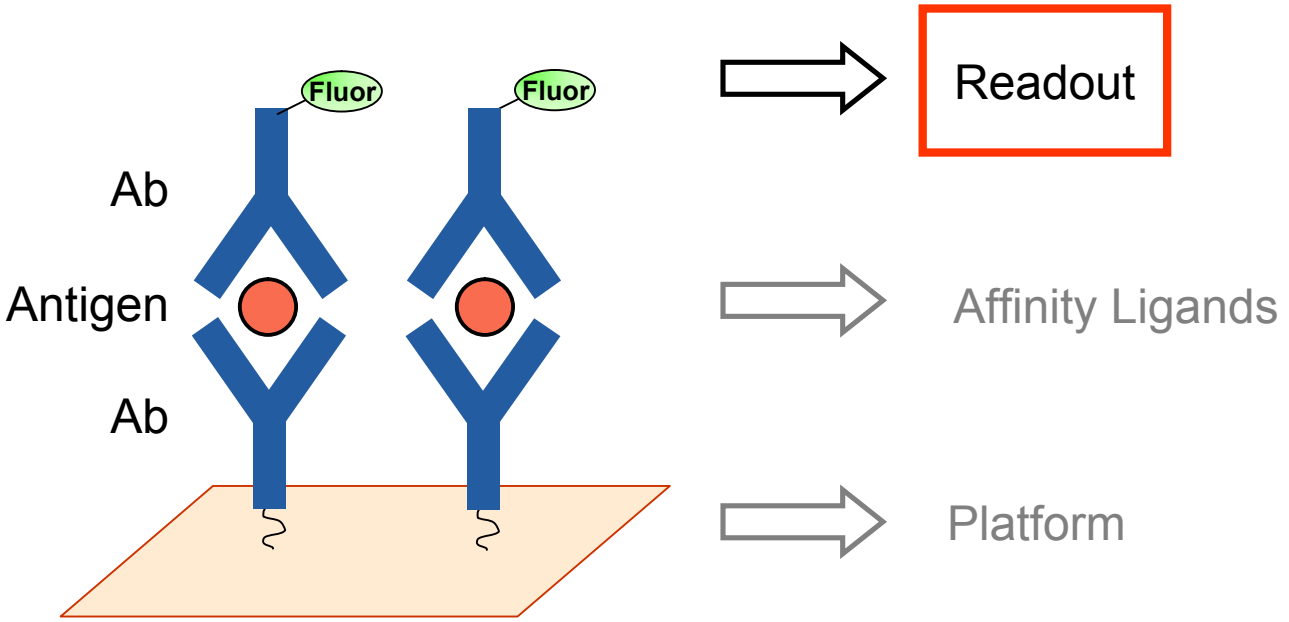
Nature **355**, 564 (1992)

Our lab has developed an efficient SELEX selection scheme using magnetic beads to partition DNA-protein complex



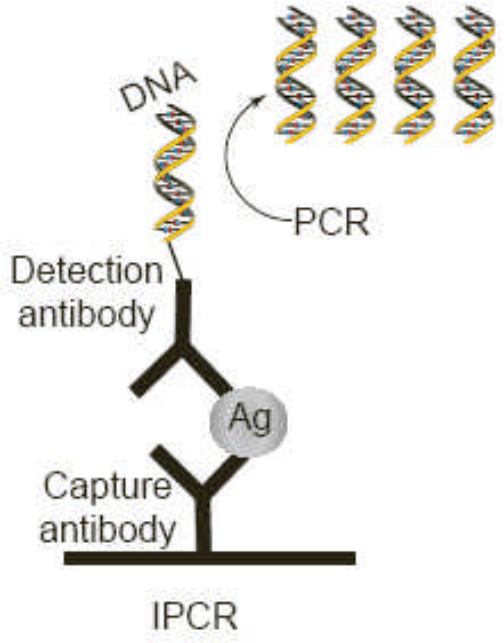
N. Fischer, C. Dolan, T. Tarasow, J. Tok, 2006

How to integrate a convenient DNA aptamer-based biodetection readout process?

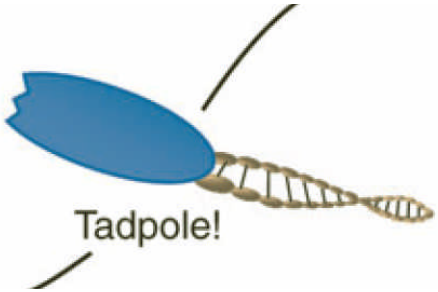


Protein detection using DNA amplification: Immuno-PCR

Exquisite levels of detection are achievable by PCR amplification



Niemeyer *et al.*, (2005) TRENDS Biotechnol.

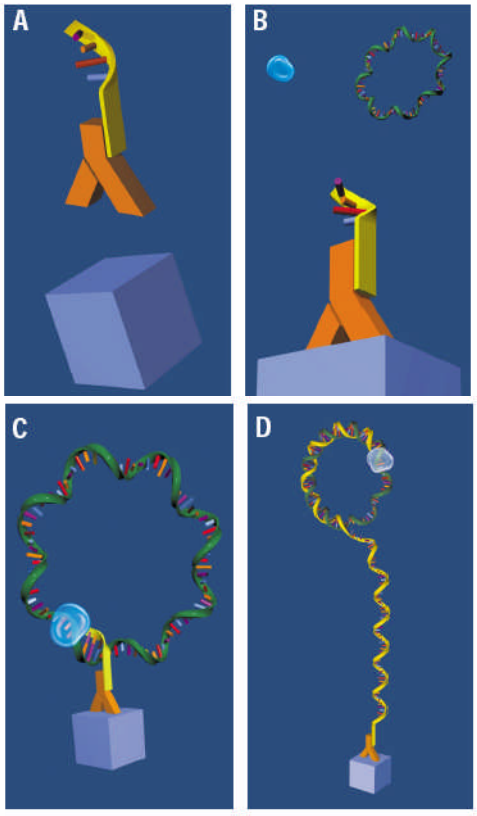


Protein-DNA Tadpoles

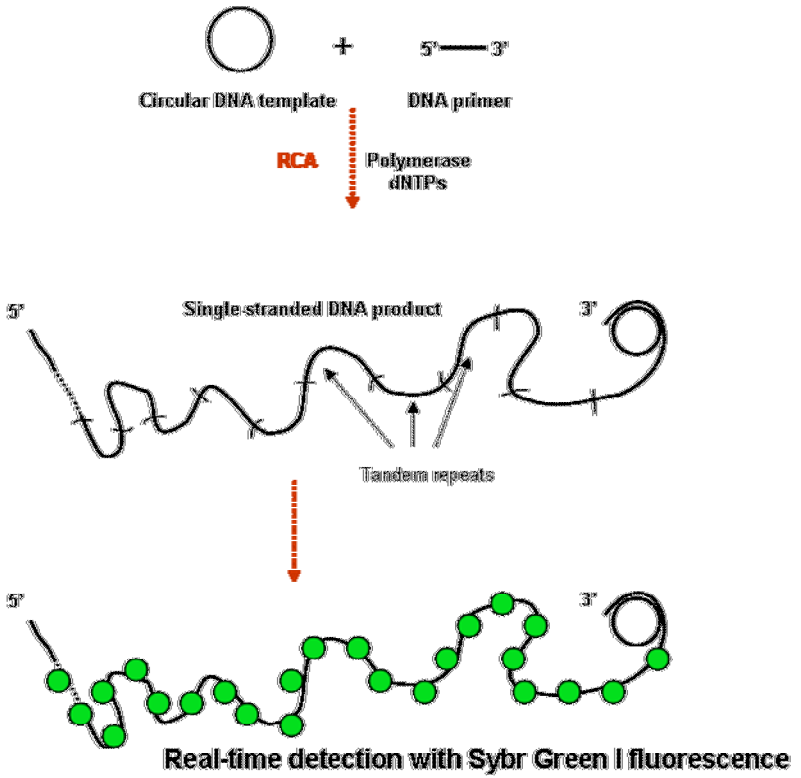
Burbulis *et al.*, (2005) Nature Methods

Synthesis of protein:DNA chimeras is labor-intensive and time-consuming

Protein detection using DNA amplification: Rolling Circle Amplification

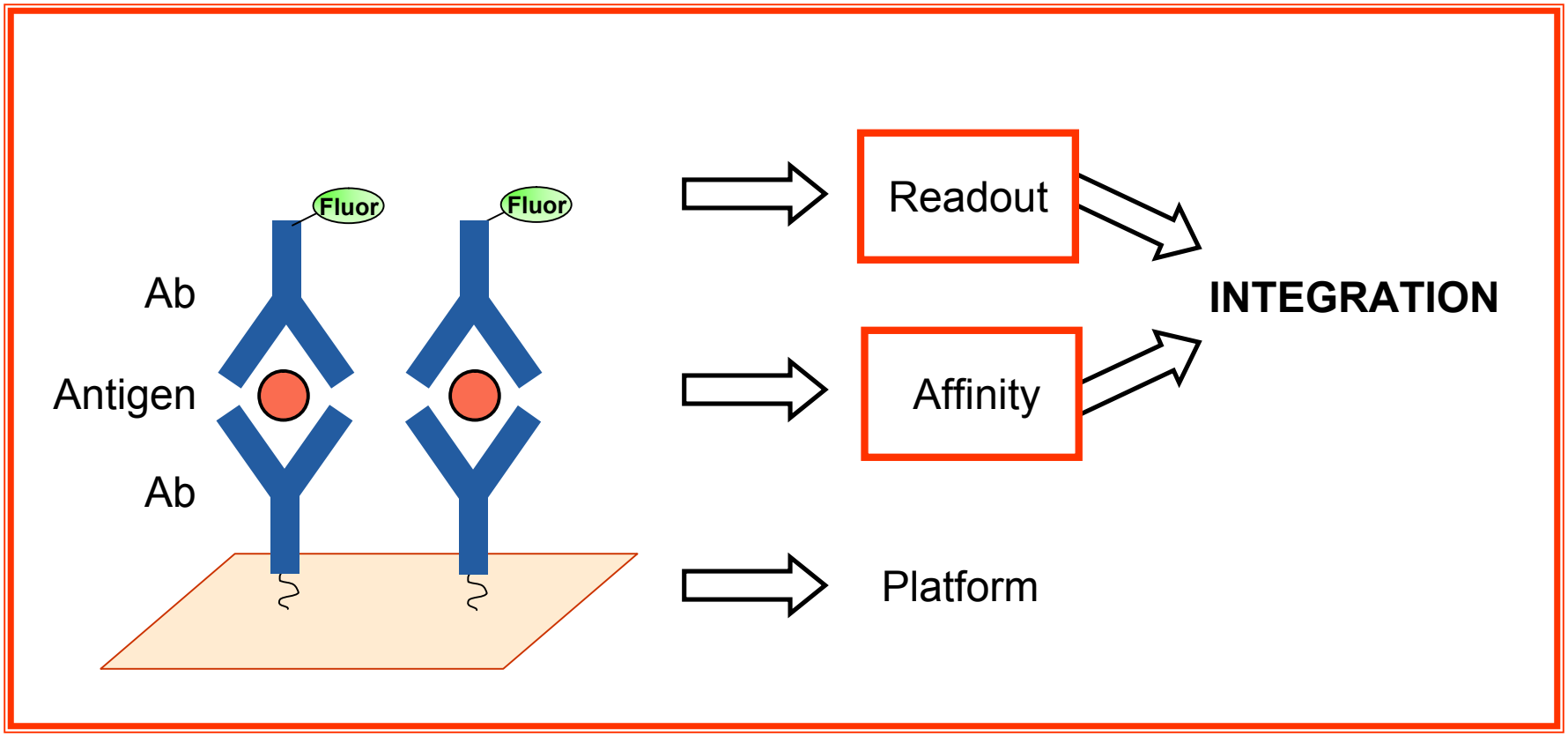


Schweitzer *et al.*, (2002) Nature Biotechnol.

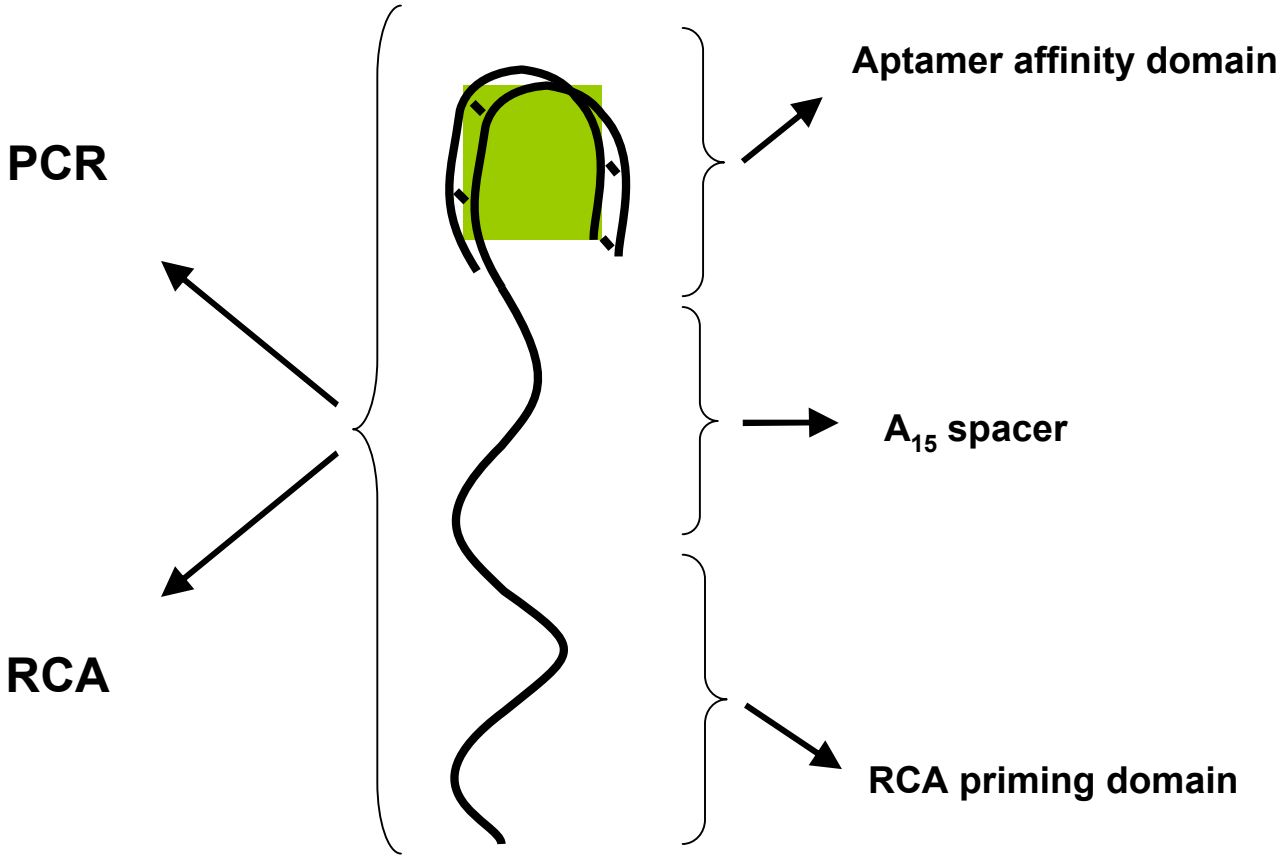


- Isothermal amplification: constant temperature (31°C)
- Product tethered to point of recognition: signal is spatially localized
- Amenable to solution or platform-based assays: high-throughput capability

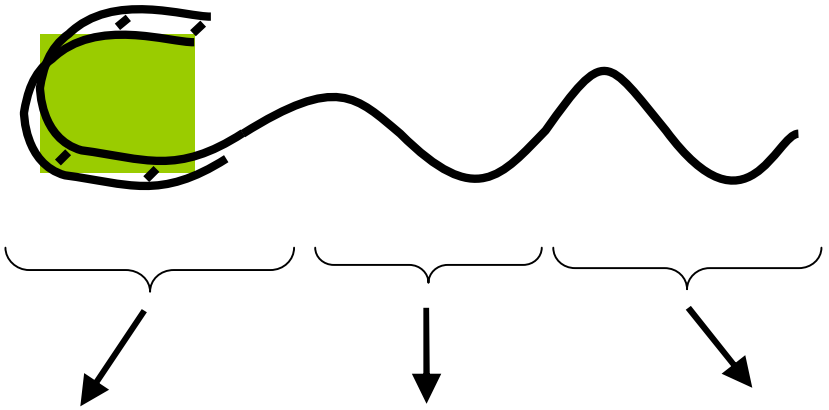
Can we integrate both affinity and readout capabilities into a single DNA molecule?



A single DNA strand with dual function: recognition and readout

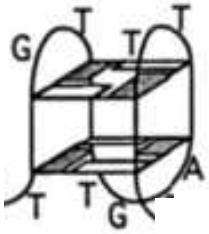


Engineered aptamer for thrombin detection



5’ – GGTTGGTGTGGTTGG – AAAAAAAAAAAAAAA - CGTGTCCTCGTTGTCTGCTC-3’

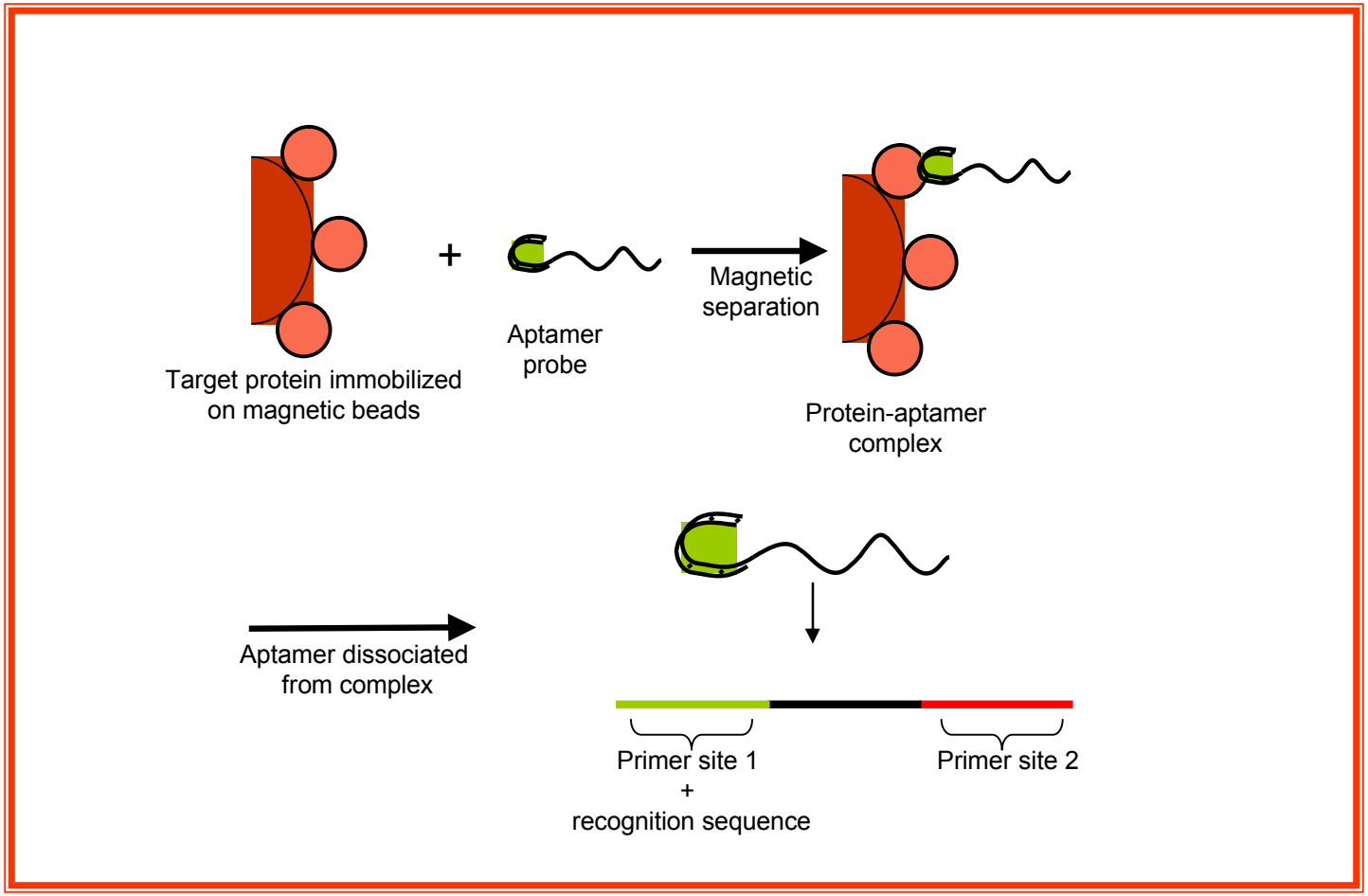
Thrombin aptamer



Bock *et al.*, (1992) Nature

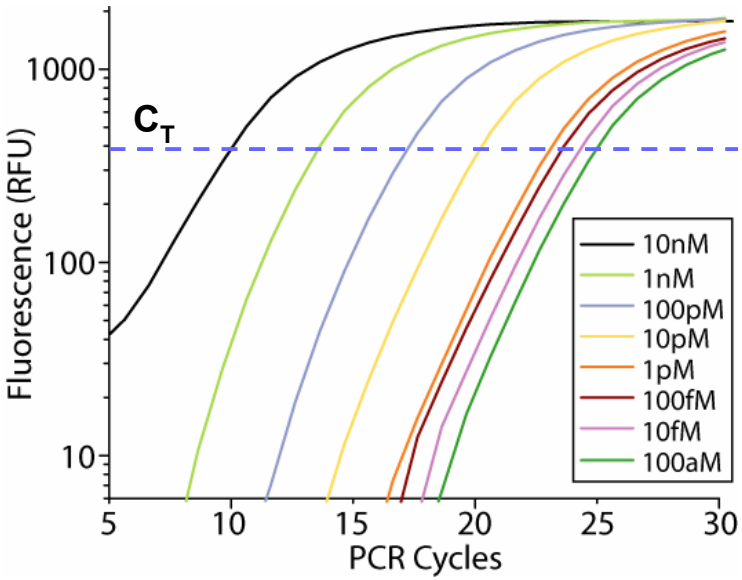
DNA molecule is only 50 bases long

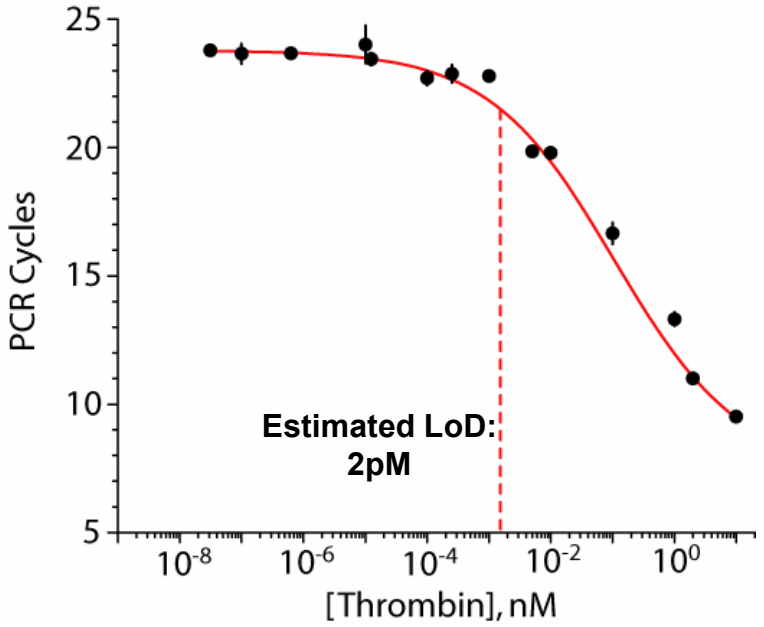
[A] Aptamer ligands can be directly detected using PCR



- Length of PCR template is minimal (50 bases)
- Convenient & efficient aptamer isolation
- Amenable for multiplex detection

PCR detection limit of thrombin is in the low pM range

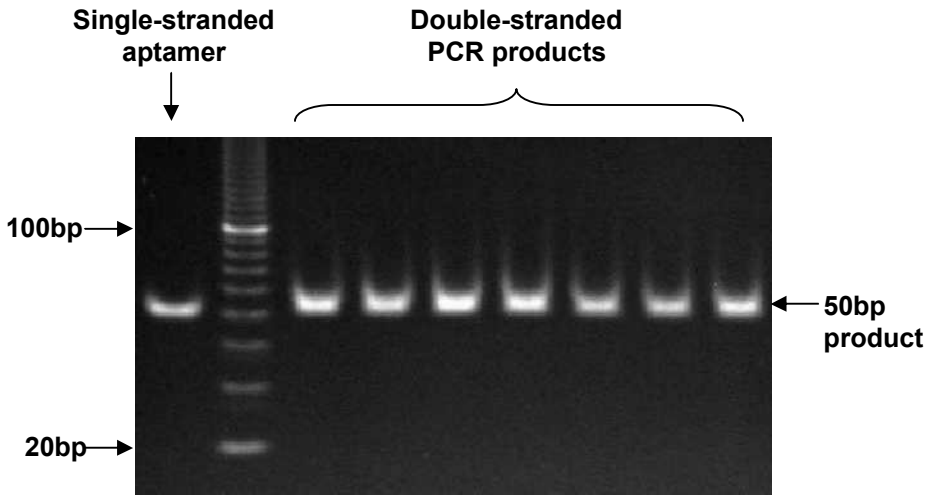


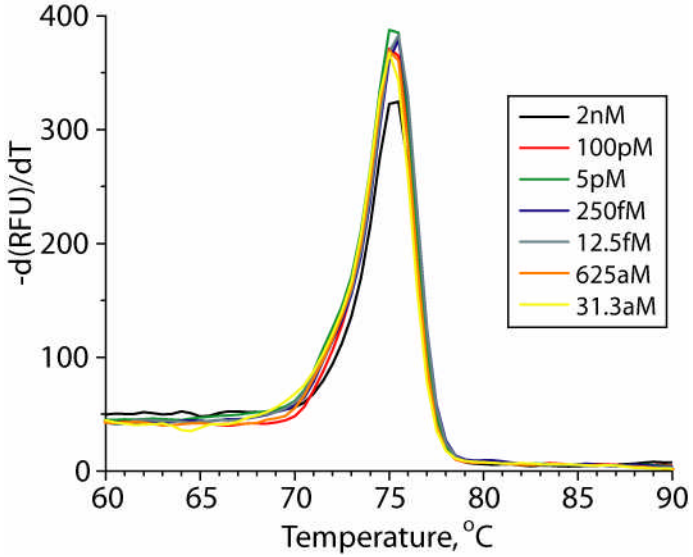


- Less than 2pM thrombin can be detected
- Detection spans 5 orders of magnitude

Fischer NO & Tok JB, 2006, Submitted

PCR product is identical to aptamer

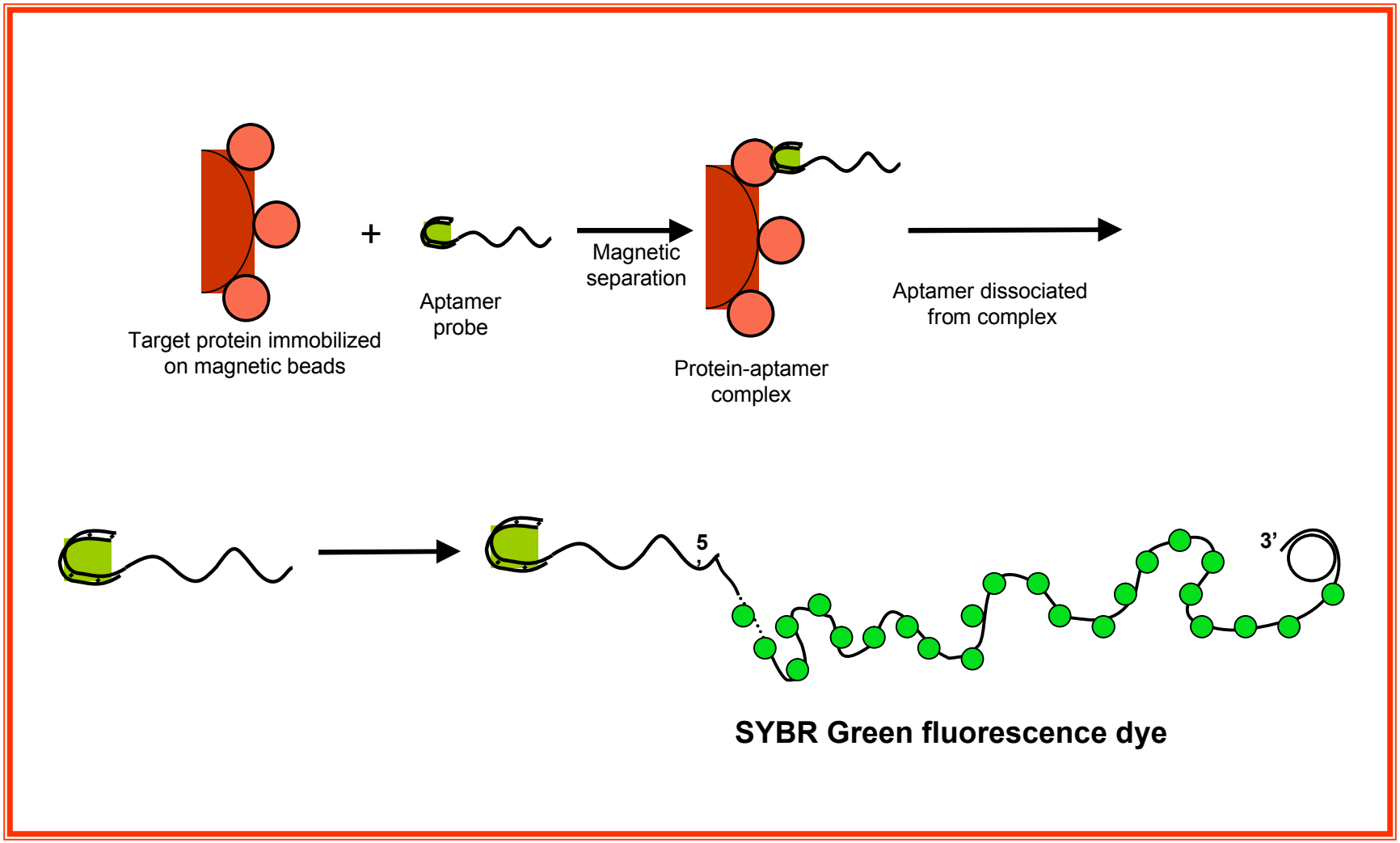




- Gel electrophoresis verifies that PCR product is same size as aptamer
- Melt curve profiles indicate presence of only one product species

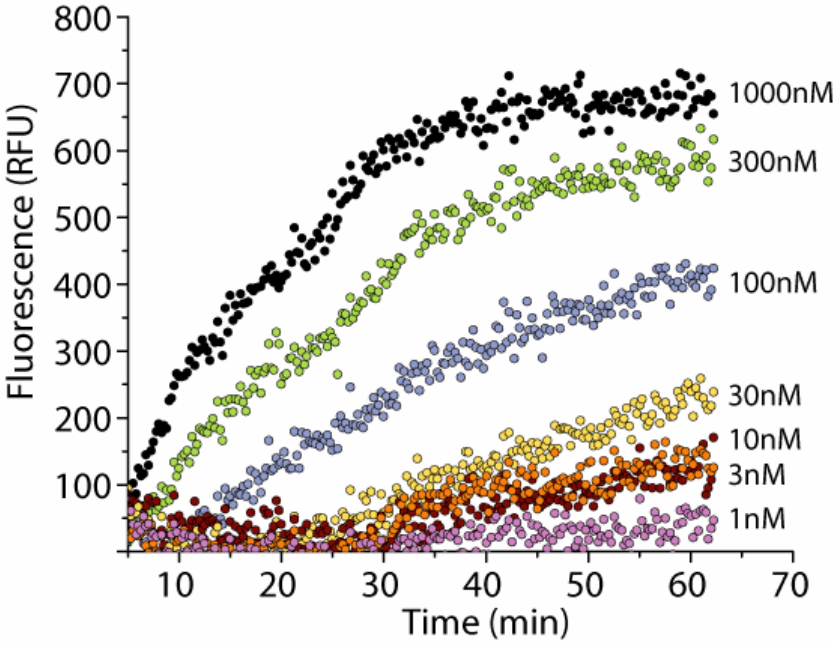
Fischer NO & Tok JB, 2006, Submitted

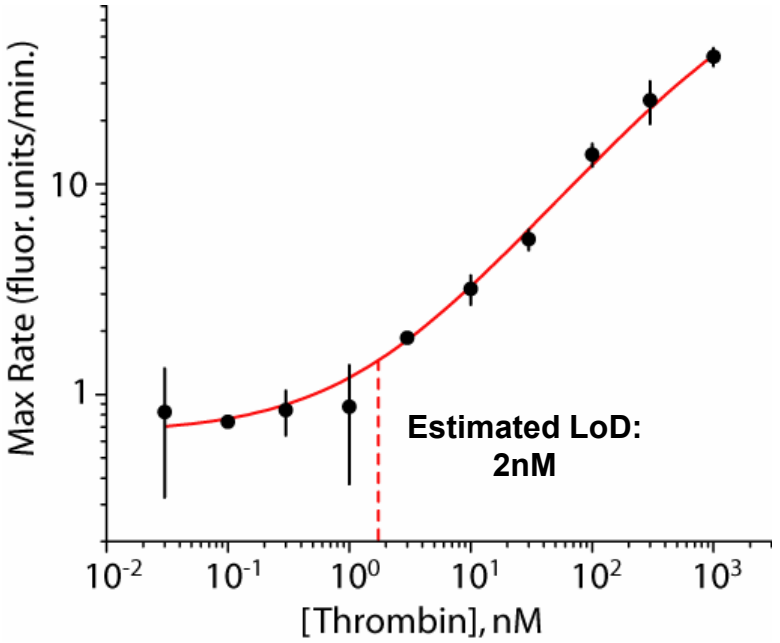
[B] Aptamer ligands can also be detected using RCA



Fischer NO & Tok JB, 2006, Submitted

RCA detection limit of thrombin is in the low nM range



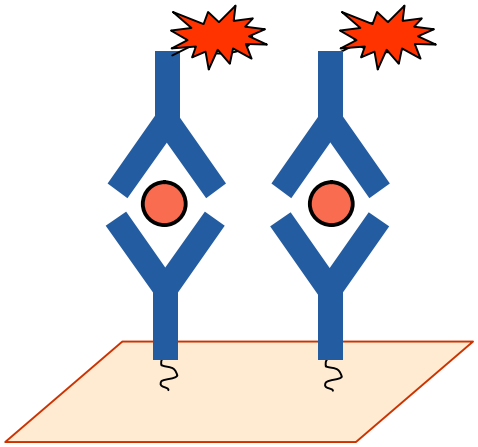


- Positive signal can be distinguished within 10 minutes
- RCA detection requires only isothermal conditions

Fischer NO & Tok JB, 2006, Submitted

Summary

- DNA aptamers are versatile molecules for affinity binding and readout



Readout

Affinity Ligands

Platform

Successful integration into a single DNA molecule

- PCR of aptamer template enables sensitive detection of protein target
- RCA is amenable to solution- and platform-based detection
- DNA aptamers are ideal affinity ligands for multiplexed detection

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Dr. Ted Tarasow
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